RESPONSE TO OFFICE ACTION

A. Status of the Claims

Claims 2 and 4-50 are pending. Claim 1 was previously canceled, claim 3 is newly canceled, claims 36-45 are withdrawn as drawn to a nonelected invention, and claim 24 is amended to more particularly point out and distinctly claim the invention.

B. Claim Objections

Claims 3 and 7 are objected to for failing to further limit claim 4. The objection to claim 3 is moot because that claim is canceled. Applicants traverse the objection to claim 7, as that claim does further limit claim 4. Claim 4 is directed to "[a] method of increasing isoflavonoid biosynthesis in a plant comprising: a)down-regulating flavanone 3-hydroxylase in said plant; and b) up-regulating isoflavone synthase by introducing a transgene encoding said isoflavone synthase into said plant." Claim 7 is directed to "[t]he method of claim 4, further defined as comprising up-regulating chalcone isomerase in said plant." Claim 7 further limits claim 4 because the method of claim 4 does not recite the up-regulation of chalcone isomerase, which is a different enzyme than the enzymes recited in claim 4 (see FIG. 1). Thus, claim 7 is more narrow than claim 4. Withdrawal of the objection to claim 7 is therefore respectfully requested.

C. **Rejections under 35 U.S.C. 103(a)**

(1) Claims 2-35 and 46-50 are rejected under 35 U.S.C. 103(a) as being unpatentable over Yu *et al.*, 2000, *Plant Physiology* 124:781-793 (Yu) in view of Wisman *et al.*, 1998, *Proc. Natl. Acad. Sci. U.S.A.* 95:12432-12437 (Wisman).

The rejection is based on the assertion that Yu teaches production of the isoflavonoid genistein in a non-legume dicot and monocot plant and plant transformed with isoflavone synthase and upregulating the phenylpropanoid pathway to increase genistein production in the presence of isoflavonoid synthase relative to a non-upregulated control. The Action acknowledges that Yu does not teach regulating flavanone 3-hydroxylase. The rejection is further based on the assertion that Wisman teaches a *tt6* mutant in *Arabidopsis* where the *tt6* gene comprises a mutant flavanone 3-hydroxylase that has lost its function of converting its substrate into product thereby allowing for the accumulation of naringenin. The rejection concludes that it would have been obvious to increase isoflavone synthase expression in a plant to increase isoflavonoid biosynthesis, in light of the teachings of Yu. The rejection further concludes that addition of the mutant flavanone 3-hydroxylase to eliminate the flavanone 3-hydroxylase branch of the pathway would be expected to lead to the accumulation of isoflavanoids due to the accumulation of their precursor naringenin.

Applicants request reconsideration and withdrawal of this rejection. Claim 4, to which claims 2 and 5-23 depend, is directed to "[a] method of increasing isoflavonoid biosynthesis in a plant comprising a) down-regulating flavanone 3-hydroxylase in said plant; and b) up-regulating isoflavone synthase by introducing a transgene encoding said isoflavone synthase into said plant." The teachings of Yu in view of Wisman do not teach or suggest that down-regulating flavanone 3-hydroxylase (F3H) would improve isoflavonoid production in a plant that is up-regulated in IFS activity.

Neither Yu nor Wisman teach or suggest that both up-regulating IFS and down-regulating F3H could achieve an increase in isoflavonoid production over IFS up-regulation alone. Indeed, neither reference establishes that down-regulation of F3H has any affect whatsoever on isoflavone biosynthesis. The ability to do so is therefore surprising and unexpected.

The phenylpropanoid pathway in plants is sufficiently complex and unpredictable such that there would not have been a reasonable expectation that down-regulating flavanone 3-hydroxylase in a plant would increase isoflavonoid biosynthesis. As further elaborated in Applicants' Response of October 28, 2008, reasons that such manipulations are unpredictable include:

- Effects of Overexpression of Phenylalanine Ammonia Lyase (PAL). PAL is the first enzyme in the phenylpropanoid pathway. Flavonoids, chlorogenic acid and lignin are all products of this pathway. Overexpression of PAL in transgenic tobacco results in a directly proportional increase in the level of chlorogenic acid, a less than proportional increase in flavonoids, and no increase in lignin (Howles *et al.*, 1996, *Plant Physiology* 112: 1617-1624, copy provided herewith). What would be predicted, however, is similar increases in all three when flux into the pathway is increased. Thus, overexpression of PAL exhibits control mechanisms that lead to unexpected downstream regulation.

- Effects of Downregulation of Caffeic Acid-3-O-methyltransferase (COMT) or Caffeoyl CoA-3-O-methyltransferase (CCoAOMT). CCoAOMT and COMT catalyze, respectively, the first and second methylation steps in the biosynthesis of lignin monomers, through the phenylpropanoid pathway. Downregulating COMT would be predicted to reduce the amount of di-methylated lignin units (which it does), but not affect the levels of mono-methylated units (or perhaps increase them). In fact, both are decreased in transgenic alfalfa expressing a COMT antisense transgene. Likewise, down-regulating CCoAOMT would be predicted to result in reductions of both mono and di-methylated lignin monomers, when in fact, in alfalfa, it only

reduces the levels of the mono-methylated units (Chen *et al.*, 2006, *Plant Journal* 48: 113-124). Thus, downregulation of COMT and CCoAOMT also exhibits control mechanisms that lead to unexpected downstream regulation.

- **Engineering Alkaloid Biosynthesis.** Various attempts at metabolic engineering in plants have lead to unexpected results. For example, genetic down-regulation of berberine bridge enzyme (BBE) or N-methylcoclaurine 3'-hydroxylase in California poppy cell cultures by antisense expression resulted in decreased growth and, surprisingly, elevated levels of several amino acids but not tyrosine, the initial precursor of the benzophenathridines. No intermediates in the alkaloid pathway were observed to accumulate in the antisense lines. Over-expression of BBE in roots led to a large increase in the levels of dihydrochelilutine, only a minor benzophenathridine in control roots.

- Metabolic Channeling. Metabolic channeling may be a common feature of plant natural product pathways, and this phenomenon, which is hard to demonstrate experimentally other than by doing flux measurements, makes it virtually impossible to predict *a priori* how a pathway will respond to changes in the levels of upstream intermediates, or how effectively a transgene product will compete for substrate from the endogenous pathway (Winkel, 2004, *Annual Review of Plant Biology* 55: 85-107).

The Action indicated that the above points were not sufficient to overcome the rejection because ". . . isoflavonoids have already been produced in non-isoflavonoid producing plants i.e. non-leguminous plants and the down regulation of flavanone-3-hydrolase did not produce any unexpected results that would render unpredictable producing isoflavonoids in transformed plants." Action at page 3. However, Applicants assert that the skilled artisan would not predict that blocking F3H would lead to an increase in isoflavonoids, since the above examples establish that much about

the control of plant metabolic pathways in general, and the flavonoid pathway specifically, is not known. Applicants also make the following points to further support this position.

Applicants first note that the Action mischaracterizes Wisman by asserting that Wisman teaches that a mutant F3S gene allows for the accumulation of naringenin. Wisman merely identified the *tt6* mutant as an F3S gene and does not teach or suggest that naringenin accumulates in that mutant and does not rule out the possibility that there is another uncharacterized enzyme that could convert naringenin to another compound. Wisman only states that "[i]n the *f3h* lines, the last enzymatic step before the branch points toward either anthocyanidins or flavonols is blocked." Wisman p. 12436, right column. Indeed, Wisman indicates that additional flavonoid pathway enzymes could be present:

> The almost unaltered quercetin level in the *fls* mutant may indicate **the presence of another related enzyme** with higher affinity for dihydroquercetin than for dihydrokaempferol as substrate.

Wisman, p. 12436, right column (emphasis added). Thus, Wisman itself provides this additional example of the unpredictability of the result of manipulation of the flavonoid pathway. The skilled artisan would therefore not be able to predict, with a reasonable expectation of success, that downregulating the F3S gene would have any effect on isoflavonoid production in an IFS up-regulated plant.

Applicants also point to Example 4 of the instant specification to further illustrate the unpredictability of metabolic engineering in the flavonoid pathways. In that example, a *tt3*/*tt6* double mutant was transformed with soybean IFS. As illustrated in FIG. 1, the *tt3*/*tt6* double mutant lacks F3H and DFR activity. Such a mutant would not be expected to produce any kaempferol or quercetin. However, both the nontransgenic *tt3*/*tt6* double mutant and the transgenic *tt3*/*tt6* double mutant expressing soybean IFS produced kaempferol and quercetin. Since all known pathways for producing kaempferol and quercetin were shut down in the *tt3*/*tt6* double mutant, there must be

unknown pathways and enzymes affecting this pathway, further establishing that there was such a lack of understanding of the pathways and control mechanisms of the flavonoid pathways that it could not be predicted that blocking F3H would lead to an increase in isoflavonoids.

Claim 24, to which claims 25-35 are dependent, is directed to "[a] transgenic plant stably transformed with a) a first selected DNA comprising a nucleic acid encoding an antisense oligonucleotide operably linked to a promoter functional in said plant, wherein said antisense oligonucleotide comprises from about 20 to about 1242 nucleotides complementary to the nucleic acid sequence of SEQ ID NO:10, from about 20 to about 815 nucleotides complementary to the nucleic acid sequence of SEQ ID NO:13 or from about 20 to about 5586 nucleotides complementary to nucleotides 82850-88437 of SEQ ID NO:15; and b) a second selected DNA comprising an isoflavone synthase biosynthesis coding sequence operably linked to a promoter functional in said plant, wherein the coding sequence encodes a polypeptide selected from the group consisting of: the polypeptide of SEQ ID NO:2, the polypeptide encoded by SEQ ID NO:3, the polypeptide encoded by SEQ ID NO:5 and the polypeptide encoded by SEQ ID NO:6." The claimed transgenic plant thus comprises a nucleic acid (a) encoding an antisense oligonucleotide that is complementary to specific sequences of plant F3H genes, and (b) comprising an IFS gene having specific sequences. Applicants assert that the plant claimed in claim 24 is not obvious for the same reasons that claim 4 is not obvious - the state of the art at the time of filing was such that there would be considerable uncertainty that combining an antisense oligonucleotide that down-regulates F3H with an IFS gene would provide increased isoflavonoid production vs. a plant comprising an IFS gene alone. As discussed above in relation to the discussion of the rejection of claim 4, the ability to predict the outcome of any particular manipulation of the flavonoid pathways is poor, since the elements that control the flux of the various metabolites, and perhaps some of the relevant enzymes themselves, have not

been identified. Further, neither of the cited references teach or suggest that the *tt6* mutant leads to the accumulation of naringenin or liquiritigenin, the substrates of F3H. Such an accumulation would not be assumed, since those compounds could be substrates for other uncharacterized enzymes.

Claim 46, to which claims 47-50 are dependent, is directed to "[a] method of increasing isoflavonoid biosynthesis in an alfalfa plant, comprising introducing into said plant a nucleic acid sequence encoding isoflavone synthase, wherein the nucleic acid sequence is operably linked to a promoter operable in said plant and wherein expression of the nucleic acid sequence results in an increase in isoflavonoid biosynthesis in the plant relative to a plant of the same genotype lacking said nucleic acid sequence." Applicants again point to the unpredictability in the art to assert that the claimed alfalfa plant is not obvious. Although Yu refers to tobacco and *Arabidopsis* plants and maize cell cultures transformed with IFS that produce genistein, the results of the claimed invention would not be expected for the following reasons.

Aside from the points made above in relation to the obviousness of the method of claims 4 and the transgenic plant of claim 24, Yu provides data to indicate that plants cannot necessarily be expected to produce isoflavonoids if transformed with an IFS gene. At pages 785-786, Yu describes the transformation of maize cells with an IFS gene. Out of 25 independent confirmed transformed lines, none produced genistein. Genistein could only be produced in the maize cells if a transcription factor that controls anthocyanin synthesis genes is expressed. Thus, the expression of isoflavonoids in maize after transformation with an IFS gene could only be achieved because the transcription factor that controls expression of anthocyanin synthesis was already known in the art. This establishes that any species could, like maize, have an element that prevents isoflavonoid biosynthesis even in the presence of the IFS gene. The skilled artisan might further expect that alfalfa would be likely to have such control elements, since related

legume species do produce isoflavonoids. Therefore, there would not be a reasonable expectation of success in increasing isoflavonoid production in any plant, and alfalfa in particular, by simply transforming the plant with an IFS gene such that IFS is expressed in the plant.

In light of the above discussion, Applicants respectfully request reconsideration and withdrawal of the rejection of claims 2-35 and 46-50 under 35 U.S.C. 103(a) as being unpatentable over Yu in view of Wisman.

(2) Claims 2-35 and 46-50 were also rejected under 35 U.S.C. 103(a) as being unpatentable over U.S. Patent 7,189,895 to McGonigle and Odell ("the '895 patent"), in view of WO 00/44909, and in further view of Applicants' asserted disclosure of the state of the prior art.

Although Applicants submitted a Declaration under 37 C.F.R. 1.131, with the Amendment and Response dated January 30, 2008, that established the currently claimed invention was made prior to the June 13, 2002 effective date of the '895 patent, the Action maintains the rejection, asserting that "the evidence submitted in the affidavit does not address all the limitations rejected under 103(a), namely the upregulation of chalcone isomerase and synthase that are taught by the '895 Patent; and thus contrary to Applicants' assertion, they had not reduced to practice the instant invention as broadly claimed."

The instant claims are directed to "[a] method of increasing isoflavonoid biosynthesis in a plant comprising: a) down-regulating flavanone 3-hydroxylase in said plant; and b) up-regulating isoflavone synthase by introducing a transgene encoding said isoflavone synthase into said plant." (Claim 4), a plant comprising transgenic plant thus comprises a nucleic acid (a) encoding an antisense oligonucleotide that is complementary to specific sequences of plant F3H genes, and (b) comprising an IFS gene having specific sequences (Claim 24), and "[a] method of increasing isoflavonoid biosynthesis in an alfalfa plant, comprising introducing into said plant a

nucleic acid sequence encoding isoflavone synthase, wherein the nucleic acid sequence is operably linked to a promoter operable in said plant and wherein expression of the nucleic acid sequence results in an increase in isoflavonoid biosynthesis in the plant relative to a plant of the same genotype lacking said nucleic acid sequence." (claim 46). Applicant's Declaration under 37 C.F.R. 1.131, dated January 30, 2008, establishes that Applicants created a plant expressing IFS in an F3H knockout background, and identified an increase in the isoflavonoid genistein prior to June 13, 2002, the priority date of the '895 patent. Thus, the '895 patent cannot be used as prior art for that teaching.

To sustain an obviousness rejection, the cited prior art references must teach or suggest all claim elements. Elements in claim 4 include the up-regulation of IFS and the down-regulation of F3H; similarly, elements of claim 24 include a plant that comprises an antisense of F3H and a transgenic IFS gene. The '895 patent is not prior art with respect to the above elements of claims 4 and 24. Additionally, WO 00/44909 does not teach or suggest down-regulating F3H. Thus, the cited art that qualifies as prior art does not teach or suggest all claim elements of claims 4 and 24. The obviousness rejection therefore cannot be sustained.

With respect to claim 46, Applicants assert that WO 00/44909 does not teach that isoflavonoid biosynthesis in an alfalfa could be increased by transforming the plant with an IFS gene with a reasonable expectation of success for the reasons discussed under **(a)** above, for example that the disclosure in Yu that isoflavonoid biosynthesis did not occur in maize cells transformed with an IFS without the expression of a transcription factor that controls anthocyanin synthesis genes preclude having a reasonable expectation of success for increasing isoflavonoid biosynthesis for alfalfa.

In light of the above discussion, withdrawal of the rejection of claims 2-35 and 46-50 under 35 U.S.C. 103(a) as being unpatentable over U.S. Patent 7,189,895 in view of WO 00/44909 is respectfully requested.

CONCLUSION

In light of the above amendments and discussion, applicants respectfully request withdrawal of all rejections and examination of withdrawn claims 36-45, since those claims are dependent on allowable claim 24.

Respectfully submitted,

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